DRUG BIOMIMETIC METABOLISM: STUDIES ON THE BENZODIAZEPINE DIAZEPAM

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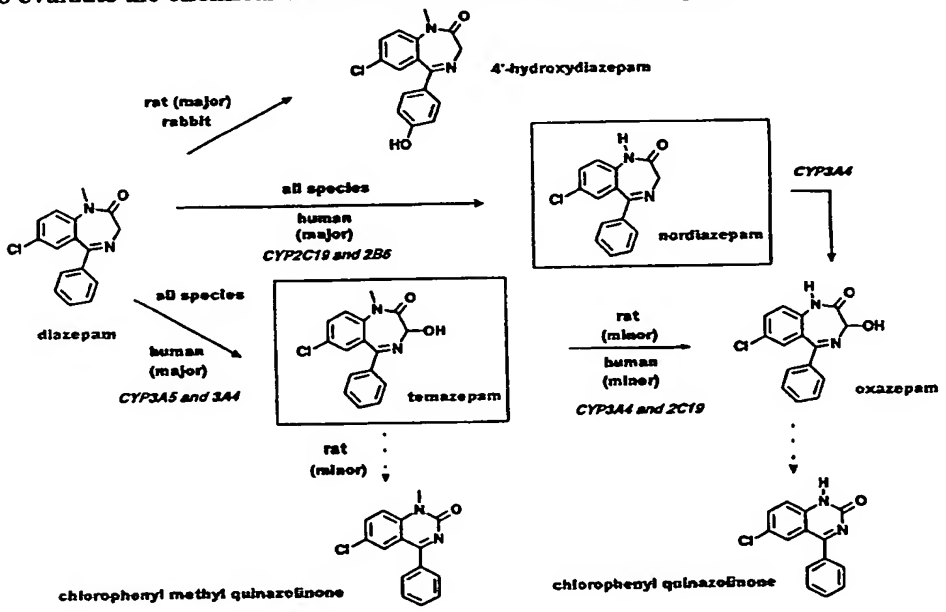
Introduction:

The study of metabolic transformation of therapeutic agents is an important part of the discovery and development process. Cytochrome P450 monooxygenases are responsible for the main oxidative pathway in drug metabolism. The active site of these enzymes, which are predominantly localized in the liver, is composed of a heme moiety. Recently, several groups have utilized metalloporphyrins as catalysts[1] in order to mimic the mechanism of cytochrome P450 and therefore mimic the oxidative metabolism of drugs. Since the metabolism of diazepam has been extensively investigated (**Scheme 1**),[2] this compound was chosen as a model to evaluate the chemical oxidative method on a benzodiazepine.



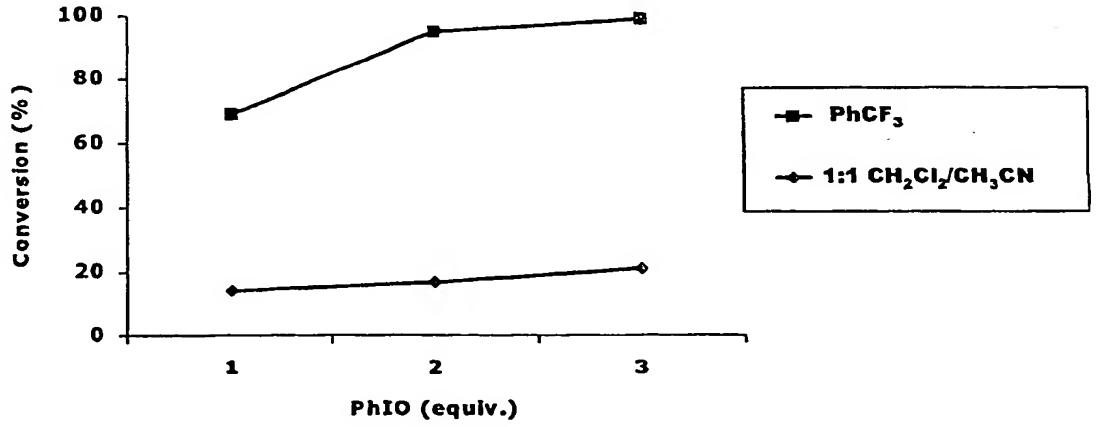
Scheme 1: Species-dependent metabolism of diazepam.

Results and Discussion:

1) Conversion

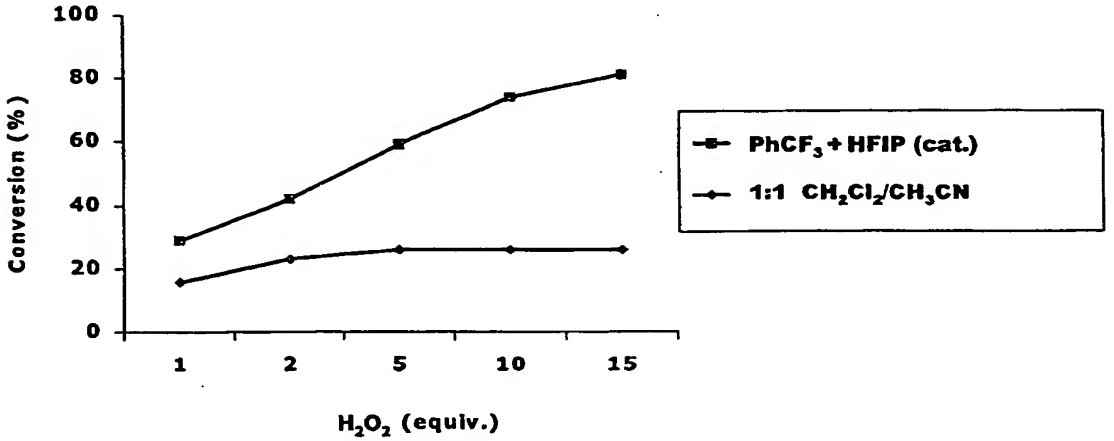
Metalloporpyhrin-catalyzed oxidation of diazepam was performed under conversion conditions (diazepam being the limiting reagent) in order to evaluate the practical aspects of the method. However, poor diazepam conversions were obtained under usual conditions[1] using CH_2Cl_2/CH_3CN as solvent. Therefore, we attempted to substitute these common solvents for a more inert fluorinated one, $PhCF_3$.[3] The novel conditions turned out to be more efficient in the

oxidation of diazepam with iodosyl[redacted]ene catalyzed by manganese and iron [redacted]kis(2,6-dichlorophenyl)porphyrin chloride (Mn(TDCPP)Cl, and Fe(TDCPP)Cl); manganese and iron tetrakis(pentafluorophenyl)porphyrin chloride (Mn(TPFPP)Cl (**Graph 1**) and Fe(TPFPP)Cl); as well as iron tetrakis(2,6-dichlorophenyl)β-octachloroporphyrin chloride (Fe(TDCPCl_8P)Cl).



Graph 1: Example of solvent effect in the oxidation of diazepam with PhIO using Mn(TPFPP)Cl as catalyst.

When hydrogen peroxide is used as oxidant, trifluorotoluene is not a good solvent compared to the commonly used solvent mixture 1:1 CH_2Cl_2/CH_3CN. This is probably due to a phase-transfer problem. However, when a co-catalytic amount of hexafluoroisopropanol (HFIP) is added to a $PhCF_3$ solution containing the usual co-catalysts ammonium acetate[4] and imidazole,[5] satisfactory conversions can be obtained in the oxidations of diazepam with an excess of hydrogen peroxide catalyzed by manganese porphyrins such as Mn(TDCPP)Cl and Mn(TPFPP)Cl (**Graph 2**).

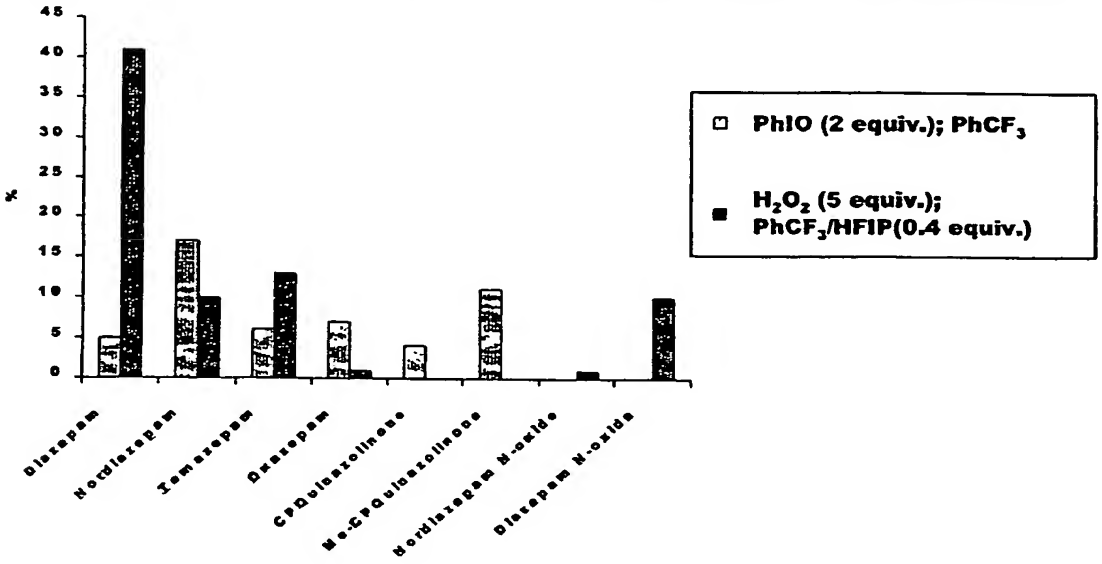


Graph 2: Example of solvent effect in the oxidation of diazepam with H_2O_2 using Mn(TPFPP)Cl as catalyst.

It should be noted that iron porphyrins are inefficient catalysts in the oxidation with hydrogen peroxide under conversion conditions. This can be explained by the high propensity of iron porphyrins to catalyze the decomposition of hydrogen peroxide.

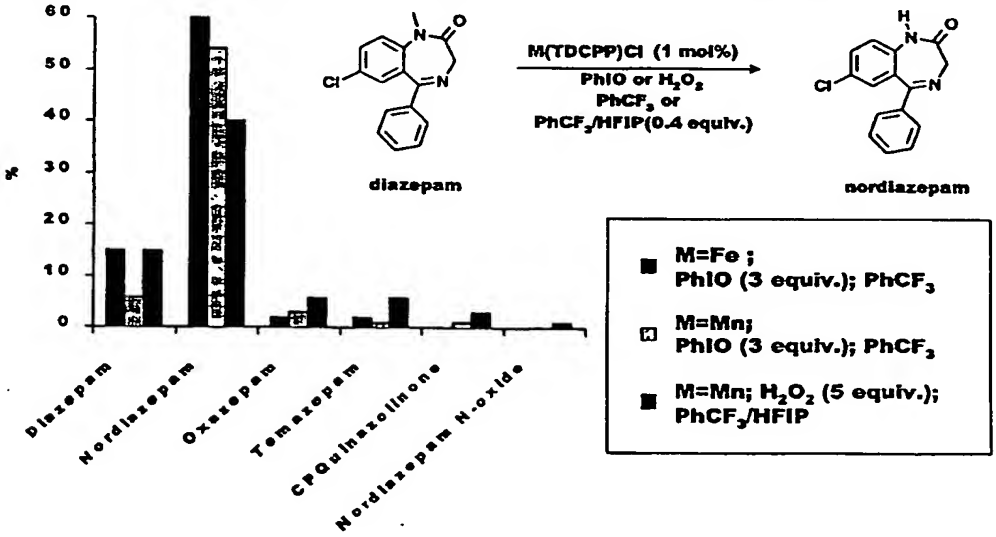
2) Chemoselectivity:

As shown in **Graph 3**, all the metabolites that had been found *in vivo* in man were obtained in the non-selective Mn(TPFPP)Cl-catalyzed oxidation of diazepam with iodosylbenzene. Surprisingly, when hydrogen peroxide is used instead of iodosylbenzene, diazepam *N*-oxide and nordiazepam *N*-oxide, compounds that were not observed in previous *in vivo* metabolism studies, are formed. Over-oxidized products, such as quinazolinones, are obtained with iodosylbenzene which provides harsher oxidative conditions compared to hydrogen peroxide.



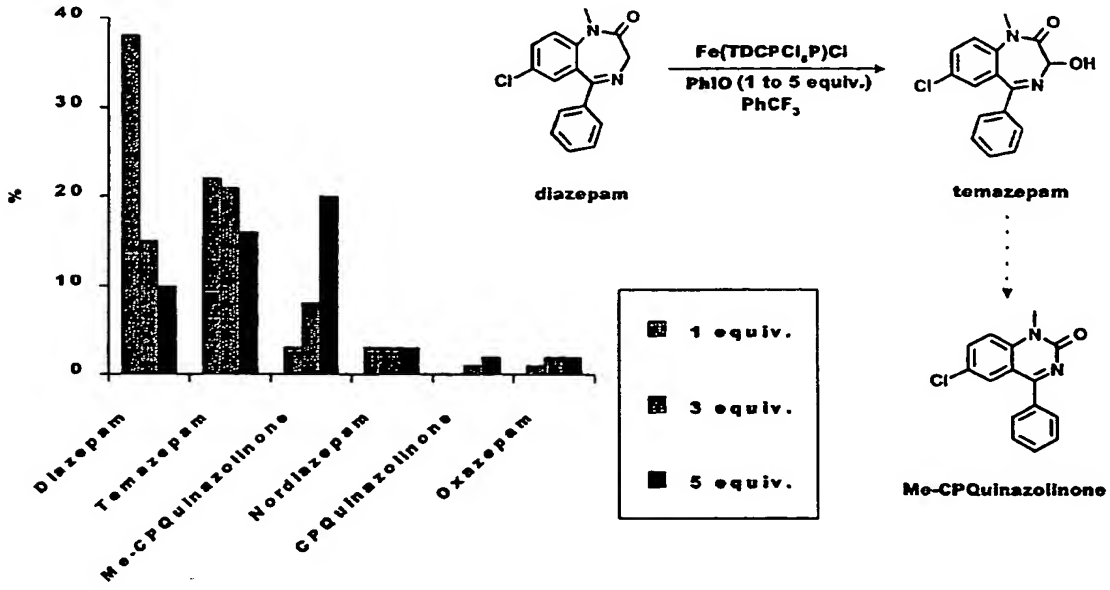
Graph 3: Mn(TPFPP)Cl-catalyzed oxidation of diazepam.

Interestingly, Mn(TDCPP)Cl and Fe(TDCPP)Cl were selective catalysts for *N*-demethylation of diazepam (**Graph 4**). A simple change into a more hindered porphyrin ligand remarkably affected the chemoselectivity of the oxidation. Fe(TDCPP)Cl-catalyzed oxidation with iodosylbenzene provides the best conditions for *N*-demethylation of diazepam. These conditions mimic CYP2C19 and 2B6 which have previously been shown to be the main cytochrome P450 enzymes responsible for *N*-demethylation of diazepam.[6]



Graph 4: Selective con[redacted]ns for *N*-demethylation of diazepam.

On the other hand, the saddle-shaped metalloporphyrin[6] Fe(TDCPCl$_8$P)Cl gives preferentially C3-hydroxylation (**Graph 5**), therefore mimicking CYP3A5 and 3A4.[6] Temazepam formed is subsequently converted to chlorophenyl methyl quinazolinone when subjected to additional iodosylbenzene.



Graph 5: Selective conditions for C3-hydroxylation of diazepam.

Conclusions:

Metalloporphyrin-catalyzed oxidation of diazepam correlates well with *in vivo* metabolism studies in man. 4'-Hydroxydiazepam, the main metabolite in rat, was not observed in these experiments. Novel conditions using fluorinated solvents have been designed so that metabolites of a benzodiazepine such as diazepam could be prepared if needed. The chemoselectivity of the reaction can be tuned by varying the porphyrin ligand of the catalyst. The general applicability of these metalloporphyrin-catalyzed oxidations is under further study.

Bibliography:

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I Claim:

1. A process for catalyzing the oxidation of organic molecules comprising contacting the molecules to be oxidized with a catalytic amount of a metalloporpyhrin and a oxidizing agent.

2. A process according to Claim 1 wherein the metalloporpyhrin is Mn(TPFPP)Cl.

3. A process according to Calim 1 wherein the metalloporpyhrin is Mn(TDCPP)Cl.

4. A process according to Calim 1 wherein the metalloporpyhrin is Fe(TDCPCl8P)Cl.

ABSTRACT

Oxidation of organic compounds is catalyzed by addition of a catalytic amount of a metalloporpyhrin